

Applicant: Takeshi SAKAMOTO, et al.

U.S.S.N. 10/805,684

Preliminary Amendment

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Amendments to the Specification

Please amend the specification as follows:

Please replace the first paragraph, which begins at line 7, with the following paragraph:

This application claims priority to US Provisional Application 60/455,766, filed March 19, 2003; US Provisional Application 60/459,936, filed April 2, 2003; and US Provisional Application 60/460,103 filed April 2, 2003. Which applications are each hereby incorporated by reference in their entirety.

Please add the following paragraph on page 1, line 10:

Sequence Listing

The instant application contains a "lengthy" Sequence Listing which has been submitted via CD-R in lieu of a printed paper copy, and is hereby incorporated by reference in its entirety. Said CD-R, recorded on August 30, 2004, are labeled "Copy 1" and "Copy 2", respectively, and each contains only one identical 896 Kb file (58770342.APP).

Please delete the paragraph starting on page 77, line 28, and replace it with the following paragraph:

The polypeptide sequence of HRMT1L1(241) set forth in Figure 50 is identical to that of HRMT1L1 (GenBank accession number NM_001535), except that the C-

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terminal 215 amino acids from 219 to 433 of HRMT1L1 are altered to “KQQSSEGDASKDTTGVLDCQQTI” (~~residues 219-241 of SEQ ID NO: 107~~) for HRMT1L1(241).

Please delete the paragraph starting on page 82, line 19, and replace it with the following paragraph:

The polypeptide sequence of mCYLN2(1047) set forth in Figure 60 is identical to that of mCYLN2 (GenBank accession number NM_009990), except that 6 amino acids from 713 to 718 of mCYLN2 are altered from “AASAEA” (~~SEQ ID NO: 160~~) to “SQHRLEL” (~~residues 713-719 of SEQ ID NO: 141~~) for mCYLN2(1047).

Please delete the paragraph starting on page 103, line 18, bridging page 104, and replace it with the following paragraph:

Generally, the expression vectors may include a promoter operably linked to a DNA encoding an interacting protein member, an origin of DNA replication for the replication of the vectors in host cells. Preferably, the expression vectors also include a replication origin for the amplification of the vectors in, e.g., *E. coli*, and selection marker(s) for selecting and maintaining only those host cells harboring the expression vectors. Additionally, the expression vectors preferably also contain inducible elements, which function to control the transcription from the DNA encoding an interacting protein member. Other regulatory sequences such as transcriptional enhancer sequences and translation regulation sequences (e.g., Shine-Dalgarno sequence) can also be operably included. Termination sequences such as the polyadenylation signals from bovine growth hormone, SV40, lacZ and AcMNPV polyhedral protein genes may also be operably linked to the DNA encoding an

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interacting protein member. An epitope tag coding sequence for detection and/or purification of the expressed protein can also be operably incorporated into the expression vectors. Examples of useful epitope tags include, but are not limited to, influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis)(**SEQ ID NO: 161**), *c-myc*, lacZ, GST, and the like. Proteins with polyhistidine tags can be easily detected and/or purified with Ni affinity columns, while specific antibodies immunoreactive with many epitope tags are generally commercially available. The expression vectors may also contain components that direct the expressed protein extracellularly or to a particular intracellular compartment. Signal peptides, nuclear localization sequences, endoplasmic reticulum retention signals, mitochondrial localization sequences, myristoylation signals, palmitoylation signals, and transmembrane sequences are example of optional vector components that can determine the destination of expressed proteins. When it is desirable to express two or more interacting protein members in a single host cell, the DNA fragments encoding the interacting protein members may be incorporated into a single vector or different vectors.

Please delete the paragraph starting on page 125, line 21, and replace it with the following paragraph:

In a specific embodiment, a protein complex used in the screening assay includes a hybrid protein as described in Section 2.1, which is formed by fusion of two interacting protein members or fragments or domains thereof. The hybrid protein may also be designed such that it contains a detectable epitope tag fused thereto. Suitable examples of such epitope tags include sequences derived from, e.g., influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis)(**SEQ ID NO: 161**), *c-myc*, lacZ, GST, and the like.

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Please delete the paragraph starting on page 127, line 20, bridging page 128 and replace it with the following paragraph:

Generally, the bait and prey vectors may include a promoter operably linked to a chimeric gene for the transcription of the chimeric gene, an origin of DNA replication for the replication of the vectors in host cells and a replication origin for the amplification of the vectors in, e.g., *E. coli*, and selection marker(s) for selecting and maintaining only those host cells harboring the vectors. Additionally, the vectors preferably also contain inducible elements, which function to control the expression of a chimeric gene. Making the expression of the chimeric genes inducible and controllable is especially important in the event that the fusion proteins or components thereof are toxic to the host cells. Other regulatory sequences such as transcriptional enhancer sequences and translation regulation sequences (e.g., Shine-Dalgarno sequence) can also be included. Termination sequences such as the bovine growth hormone, SV40, lacZ and AcMNPV polyhedral polyadenylation signals may also be operably linked to a chimeric gene. An epitope tag coding sequence for detection and/or purification of the fusion proteins can also be incorporated into the expression vectors. Examples of useful epitope tags include, but are not limited to, influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis)(**SEQ ID NO: 161**), *c-myc*, lacZ, GST, and the like. Proteins with polyhistidine tags can be easily detected and/or purified with Ni affinity columns, while specific antibodies to many epitope tags are generally commercially available. The vectors can be introduced into the host cells by any techniques known in the art, e.g., by direct DNA transformation, microinjection, electroporation, viral infection, lipofection, gene gun, and the like. The bait and prey vectors can be maintained in host cells in an extrachromosomal state, i.e., as self-replicating plasmids or viruses. Alternatively, one or both vectors can be integrated into chromosomes of the host cells by conventional techniques such as selection of stable cell lines or site-specific recombination.

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Please delete the paragraph starting on page 135, line 16, and replace it with the following paragraph:

Many different types reporters are useful in the screening assays. For example, a reporter protein may be a fusion protein having an epitope tag fused to a protein. Commonly used and commercially available epitope tags include sequences derived from, e.g., influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis)(~~SEQ ID NO: 161~~), *c-myc*, lacZ, GST, and the like. Antibodies specific to these epitope tags are generally commercially available. Thus, the expressed reporter can be detected using an epitope-specific antibody in an immunoassay.